

### Abstract

Estimation of lithium in 24 samples of serum and saliva show that there is a very high correlation between the lithium levels in serum and saliva ( $r = +0.88$ ). The ratio found in our patients is very similar to that reported from North America. Estimation of lithium in saliva at different periods of time show there is a good degree of stability for saliva lithium levels. Thus saliva can be used as a substitute for serum for lithium monitoring. The practical advantages of this are discussed.

### ANNEX B: Examples

#### New Example 1:

##### Preparation of luminol concentrated stock

The following mixture was prepared in a disposable polystyrene test tube:

100 $\mu$ L of Sodium Luminol stock solution of 40mg/mL H<sub>2</sub>O

14.6 $\mu$ L of p-iodophenol stock solution of 100mg/mL DMSO

1886 $\mu$ L H<sub>2</sub>O

The mixture was stored at 4°C until use.

#### New Example 2:

##### Preparation of luminometric enzyme and determination of glucose

The following mixture was prepared in a disposable polypropylene test tube:

0.6 mL Glucose Oxidase stock of 4400U/mL H<sub>2</sub>O

1.2 mL Horseradish Peroxidase stock of 1100U/mL H<sub>2</sub>O

0.6 mL 0.5M Tris-HCl, pH 8.5

2.6 mL isotonic Sodium Fluoride solution (1.75%w/v in H<sub>2</sub>O)

625 $\mu$ L 20%w/v Bovine Serum Albumin (Cohn's Fraction V) in isotonic Sodium Fluoride solution

### 625 $\mu$ L Luminol concentrate

For test: 10 $\mu$ L sample was placed in the bottom of a Microfuge tube. The tube was inserted into the counting chamber of a Labsystems Luminoskan TL Plus and its luminescence counted for 1 second (= background luminescence). 45 $\mu$ L of the luminometric enzyme mix was added to the test tube, the lid of the counting chamber was lowered into closed position and counting proceeded for 1 second. The background luminescence was deducted from the chemiluminescence of the enzymatic reaction.

### New Example 3:

#### Preparation of Reagents for the Luminometric Determination of Hemoglobin

##### Component A:

The following solutions were mixed in a sterile disposable polypropylene test tube:

- 0.06 mL Luminol concentrated stock (Example 1)
- 0.60 mL 0.5M TRIS HCl buffer, pH 8.5
- 0.30 mL 0.25M di-sodium EDTA
- 2.04 mL isotonic sodium fluoride (1.75%w/v in water)

##### Component B:

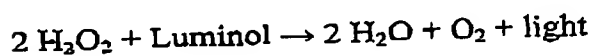
The following solutions were mixed in a sterile disposable test tube:

- 0.30 mL 0.25M di-sodium EDTA
- 0.171 mL 35% H<sub>2</sub>O<sub>2</sub>
- 2.529 mL water

### New Example 4:

#### Luminometric Determination of Hemoglobin

Hemoglobin was determined by its peroxidase-like activity, i.e. its ability to catalyze the following reaction:



10 $\mu$ L sample was placed in the bottom of a Microfuge tube. The tube was inserted into the counting chamber of a Labsystems Luminoskan TL Plus. 75 $\mu$ L of Component A were added to the tube and its luminescence counted for 1 second (= background luminescence). Then, 75 $\mu$ L of Component B were added to the tube and its chemiluminescence counted for 1 second. The background luminescence was deducted from the chemiluminescence of the hemoglobin reaction.

### New Example 5:

#### Glucose and Hemoglobin in Hair Shafts and Correlation with blood

A glucose loading test was conducted. Under overnight fasting conditions a capillary blood specimen was taken and its glucose levels was determined by a Bayer Glucometer Elite. At the same time, about 5 –10 hair strands were from the forearm and inserted into 200 $\mu$ L of distilled water. 10 $\mu$ L aliquots from this solution were tested for glucose and hemoglobin, as described above. Thereafter the glucose level in blood was raised by the ingestion of more than 50 grams of glucose, followed by a repeat blood glucose test and a hair glucose and hemoglobin test.

The hemoglobin level of the hair specimen is employed as a correction factor, representing the amount of blood. Therefore, the glucose chemiluminescence counts were divided by the hemoglobin chemiluminescence in order to arrive at a value, which represents the amount of glucose in a unit of hair blood.

The results are presented in Table 1:

Table 1

#### Glucose and Hemoglobin in Hair and Blood

Blood Glucose	Hair Glucose Counts	Hair Hemoglobin Counts	Glucose/Hemoglobin Ratio in Hair
87 mg/mL	6606	2644	2.49
123 mg/mL	2731	902	3.03

Conclusions:

1. The results demonstrate that the level of glucose in hair, as corrected by the level of hemoglobin, correlates with the level of glucose in blood.
2. Thus, the invention can be used to determine the glucose level in blood, without the need to obtain blood. Plucked hair, as an example for a non-blood specimen, which nevertheless contains blood, can serve as the specimen for that purpose.